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Thomsen-Friedenreich Antigen (TF-Ag) is a carbohydrate adenocarcinoma-associated antigen. TF-Ag expression contributes to breast cancer cell adhesion and metastasis. Antibody to TF-Ag developed in our lab blocks metastatic interactions and improves survival in a mouse metastatic breast cancer model. Immunizations with peptide mimics of carbohydrate antigens have been shown by others to create immune responses to the carbohydrates. Our hypothesis is that vaccinations using peptide mimics of TF-Ag will generate immune responses to TF-Ag on tumor cells which will be useful in active immunotherapy of cancer.

To characterize the ability of the peptide to mimic TF-Ag, *in vitro* studies including immunoblotting, inhibition ELISA, and Biacore analysis were performed and all exhibited successful mimicking. An *in vitro* metastasis model showed significant inhibition of cancer cell adhesion using the peptide mimics. In *in vivo* studies, peptide mimics were used to immunize rabbits and mice, and serum was analyzed for TF-Ag antibodies. Initial vaccinations did not yield antibodies reactive to TF-Ag, therefore improvements will be made to the immunization protocol. X-ray crystallography and computer modeling will be utilized to generate better peptide mimics. This research can be clinically applied to decrease tumor burdens and improve current therapeutic regimes for breast cancer.

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Annual Report

Introduction

The purpose of this study is to expand our understanding and utilization of the Thomsen-Friedenreich Antigen (TF-Ag) which is a carbohydrate tumor-associated antigen highly expressed on several types of adenocarcinoma cells, including breast tumor cells (1,2,3). TF-Ag expression contributes to cancer cell adhesion and its metastasis to non-breast tissue (1,2,3). Passive transfer of our antibody to TF-Ag, JAA-F11, creates a survival advantage in a mouse metastatic breast cancer model and decreases human cancer cell adhesion in 3 different *in vitro* models of metastasis. These results indicate that a patient's immune response to TF-Ag could also create a survival advantage. Our goal is to utilize a peptide mimic of TF-Ag to create an improved immune response to the antigen. This response could potentially allow the immune system to target and destroy TF-Ag expressing cancer cells.

Body

My training and research accomplishments for the past year have focused on the peptide mimic of TF-Ag. Following the outline of the approved Statement of Work (SOW), my research was to be centered on a vaccination scheme with the peptide mimic and the analysis of the antibody response to the mimic. The peptide mimic was linked to a carrier protein to increase antigenicity and mixed with an adjuvant for immunizations of mice. The vaccinations took place over a 2 ½ month period. The results of these experiments were not as successful as hoped. The mouse sera was tested for antibodies to the peptides themselves, and antibody was present, showing that an immune response was effectively created during the immunizations. However, the important question is whether the elicited antibodies can bind to TF-Ag, the antigen being mimicked. The serum was first tested for IgG antibodies that reacted with TF-Ag. Twelve of fourteen mice showed no antibody response, while two mice showed a very low level of reactivity. In the control mice, one of seven showed a positive response. Variability was seen when the assay was repeated. The serum was then tested for IgM antibodies that reacted with TF-Ag. A small increase was seen in the post-immune sera compared to the pre-immune, showing that there was an increase in antibody levels, however the increase was minimal and may not be characterized as a good antibody response.

Services provided by Sigma Genosys were also used to prepare the peptide mimics and perform rabbit immunizations, in hopes that this different system would provide better results. Four rabbits were immunized with peptide mimics, and one of these rabbits showed a low level increase after 3 immunizations. The immunizations were continued on this rabbit, however no further increase in antibody level was seen.

The technical and unexpected difficulties in these experiments caused several time-consuming digressions from the previously determined path. There may have been problems with the chemical coupling of the peptide to the carrier molecule used for immunizations, causing the conformation of the peptide to change. This could have led to the production of antibodies that were not reactive to TF-Ag itself. Without successful

antibody production, Task 1c. of the SOW could not be performed. Blood draws also yielded minimal amounts of serum for testing.

Deviations from the SOW due to the difficulties that have occurred are as follows:

(1) Mouse sera will be drawn after each immunization, not just at the end of the immunization scheme, as to not miss any fluctuations in antibody levels.

(2) More sera will be drawn so that several experiments can be performed on the sera. A different blood drawing method may be employed.

(3) 4T1 cells instead of JC cells will be used because they were determined experimentally to be a better model of breast cancer, as the cancer and its metastases progress similarly to human cancers (4,5). The passive transfer experiments have been performed already using 4T1 cells so this study can be used as a comparison and to ensure that the data is repeatable.

(4) Immunizations were and will be performed by subcutaneous injection of the immunization mixture as this is the preferred and recommended protocol. Mammary fat pad injections will only be used when injecting tumor cells to establish primary tumors as stated in Task 3.

(5) Different adjuvants will be tested for their efficacy in boosting the immune response.

(6) The experiments focused on one peptide mimic of TF-Ag that exhibited the best results in the first *in vitro* experiments. However, there are two other sequences that can be tested that may still act as mimics when used *in vivo*.

Additional *in vitro* characterizations have been performed showing that the peptides do in fact mimic TF-Ag. These include an *in vitro* adhesion model in which the peptides were tested for their ability to inhibit the binding of cancer cells to vascular endothelial cells (3). The endothelial cells express a ligand known to bind TF-Ag called galectin-3 (3). The data showed that the mimic inhibited the rolling adhesion of the cancer cells by 50% and the stable adhesion by 81%. Even though this is considered an *in vitro* experiment, the ability to inhibit the binding of live cancer and endothelial cells implies that the peptide mimic will be beneficial when used *in vivo*.

In vitro, we have demonstrated by several methods that the peptide can mimic TF-Ag. However, this mimicking ability has not translated *in vivo*. This has led us to formulate additional experiments that will create an improved peptide mimic. Currently, our antibody to TF-Ag is being purified for use in X-ray crystallography to visualize the specific interactions of the peptide and antibody. This will allow for changes in the peptide sequence to be made to improve the fit of the antibody with the peptide, in hopes that this will enhance the *in vivo* response. Computer modeling is also being explored to predict how specific changes in the peptide sequence will alter the antibody binding to the peptide. This will allow us to produce new or improved peptide sequences for *in vivo* use.

The technical and unexpected difficulties were numerous and created barriers to moving forward with the next steps as outlines in the SOW. However, additional information was gained that is leading to new experimental avenues and possibilities, and will lead to further characterization of this peptide mimic, promoting a better understanding of its capabilities in creating an improved immune response to TF-Ag bearing breast tumor cells.

Key Research Accomplishments

List of key accomplishments:

- Immunoblot experiments showed that antibody to TF-Ag (F11) binds the peptide mimic.
- Biacore experiments provided an affinity of F11 for the peptide: 5.73×10^{-4}.
- Inhibition ELISA experiments showed that the peptide can inhibit F11 binding to TF-Ag by up to 50%.
- *In vitro* adhesion assays demonstrated a significant inhibition of the adhesion of cancer cells to vascular endothelium by the peptide mimic.
- Immunizations with the peptide mimic successfully generated antibodies to the peptide mimic, showing that the vaccination scheme is working.

Reportable Outcomes

List of reportable outcomes:

Poster Presentations:

- J. Heimburg, A. Almogren, S. Morey, O.V. Glinskii, V.H. Huxley, V.V. Glinsky, R. Roy, R. Cheng, K. Rittenhouse-Olson. Development and Characterization of a Peptide Mimic of T-Antigen. Presented on November 20th 2004 at the Joint Meeting of the Society for Glycobiology and the Japanese Society for Carbohydrate Research, Honolulu, Hawaii.

Abstracts:

- J. Heimburg and K. Rittenhouse-Olson. Active Immunization Using a Peptide Mimic of a Carbohydrate Tumor Antigen. Submitted December 15th 2004 for the Era of Hope Department of Defense Breast Cancer Research Program Meeting.
- J. Heimburg, A. Almogren, S. Morey, O.V. Glinskii, V.H. Huxley, V.V. Glinsky, R. Roy, R. Cheng, K. Rittenhouse-Olson. Development and Characterization of a Peptide Mimic of T-Antigen. *Glycobiology* v14:1159, 2004.

Presentations:

- K. Rittenhouse-Olson. T-Antigen Potential for Clinical Applications. National Cancer Institute, February 2004. This presentation included data on the peptide mimic of T-Antigen supported by this grant.
- K. Rittenhouse-Olson. Carbohydrates in Drug Development. Cambridge Health Institute's 2nd Annual Glycomics Conference, April 2004. This presentation included data on the peptide mimic of T-Antigen supported by this grant.
- K. Rittenhouse-Olson. Attempts to Create Immune Responses to T-Antigen, a Carbohydrate Tumor-Associated Antigen. National Institute of Allergy and Infectious Disease, October 2004. This presentation included data on the peptide mimic of T-Antigen supported by this grant.

A manuscript for submission to *Glycobiology* is currently in progress.

No development of cell lines/tissues/etc, animal models, additional funding, or employment has yet occurred as a result of work performed under this grant.

Conclusions

The objective of the BCRP is to promote innovative research directed toward eradicating breast cancer while preparing promising graduate student for careers in breast cancer research through successful training and research accomplishments. I feel that while working on this research, I have encountered various obstacles and setbacks that have required deviations from the outlined SOW. However, these obstacles have allowed me to explore new ideas and have fostered greater independent thinking to create solutions to the problems. I have reached certain goals in the research project thus far and will continue to reach new goals while completing the remaining tasks. I feel prepared to deal with the adversities that occur during scientific research and this will propel my training to higher levels while preparing me for a successful career in breast cancer research.

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